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Lab Resource: Stem Cell Line

Generation of human embryonic stem cells from abnormal blastocyst diagnosed with adrenoleukodystrophy

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ABSTRACT

Human embryonic stem cell (hESC) line *ch*HES-480 was derived from abnormal blastocyst diagnosed with adrenoleukodystrophy (ALD) after preimplantation genetic diagnosis (PGD) treatment. DNA sequencing analysis confirmed that *ch*HES-480 cell line carried a hemizygous missense mutation c.1825G>A(p.Glu609Lys) of *ABCD1* gene. Characteristic tests proved that the *ch*HES-480 cell line presented typical markers of pluripotency and had the capability to form the three germ layers both *in vitro* and *in vivo*.

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Resource table:

Name of Stem Cell line	*ch*HES-480
Institution	National Engineering and Research Center of Human Stem Cell
Person who created resource	Qi Ouyang
Contact person and email	Qi Ouyang: loretta0730@163.com
Date archived/stock date	Sep 24, 2015
Origin	Abnormal blastocyst from adrenoleukodystrophy
Type of resource	Human embryonic stem cells derived from PGD–analyzed abnormally blastocyst
Sub-type	Cell line
Key transcription factors	*POU5F1*, *SOX2*, *NANOG*, *KLF4*, *TERF1*, *THY1*
Authentication	Identity and purity of cell line confirmed (Fig. 1)
Link to related literature	N/A
Information in public databases	N/A
Ethics	The study was approved by the ethical committee of Reproductive and Genetic Hospital of CITIC-Xiangya and informed consent was obtained from the patient.

Resource details

Human embryonic stem cell (hESC) line *ch*HES-480 was derived from abnormal blastocyst diagnosed with adrenoleukodystrophy (ALD) after preimplantation genetic diagnosis (PGD) treatment. DNA sequencing analysis confirmed a hemizygous missense mutation c.1825G>A(p.Glu609Lys) of *ABCD1* gene in the cells (Fig. 1A). This mutation was already documented in the main databases such as HGMD (http://www.hgmd.cf.ac.uk/ac/search.php) and ClinVar (http://www.ncbi.nlm.nih.gov/clinvar), indicating a pathogenic effect of this nucleotide change. The *ch*HES-480 cells displayed pluripotent cell morphology (Fig. 1B), and expressed pluripotency related genes such as *POU5F1*, *SOX2*, *NANOG*, *KLF4*, *TERF1* and *THY1* (Fig. 1C). The *ch*HES-480 cells were also positive for pluripotent markers including OCT4, NANOG, TRA-1-60 and TRA-1-81 as well as alkaline phosphatise (Fig. 1D) and showed a stable Karyotype of 46, XY during prolonged culture by the karyotype analysis (Fig. 1E). The *ch*HES-480 cells had the capability to differentiate into the derivatives from all the three germ layers both *in vitro* (Fig. 1F) and *in vivo* (Fig. 1G).

Materials and methods

Source of embryo

This study was approved and guided by the local ethical committee of Reproductive and Genetic Hospital of CITIC-Xiangya. Abnormal embryos with aberrant mutant of *ABCD1* gene were donated by a couple of adrenoleukodystrophy carriers after signed informed consent.

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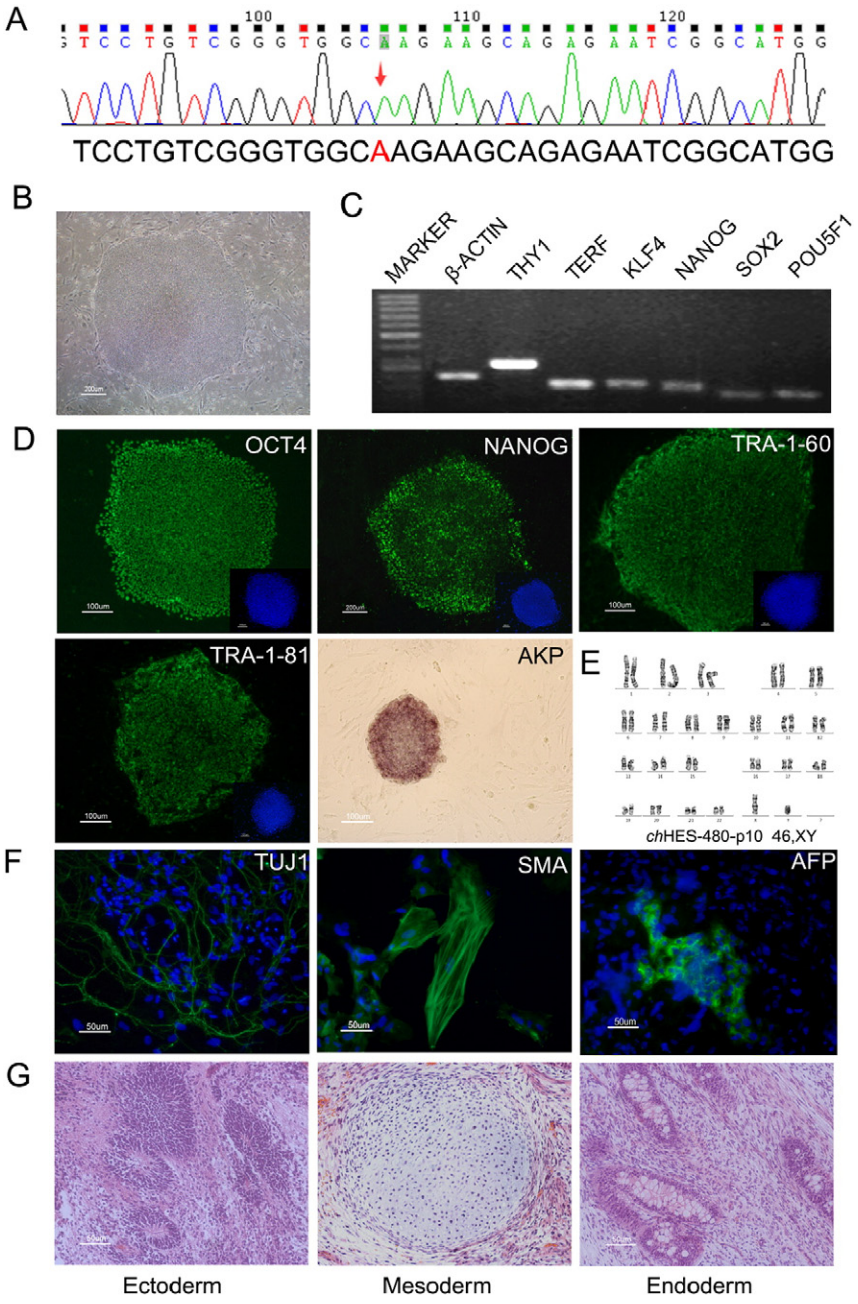

Fig. 1. Characterization of *ch*HES-480 cells. (A) A hemizygous mutation (red arrow), c.1825G>A(p.Glu609Lys), of *ABCD1* gene was identified in *ch*HES-480 cell line; (B) Cells showed typical round shape colonies on the feeder layer of MEF; (C) Pluripotent genes detected by RT-PCR were expressed in *ch*HES-480 cells including *POU5F1*, *SOX2*, *NANOG*, *KLF4*, *TERF1* and *THY1*; (D) Undifferentiated Colonies were positive for the pluripotency markers of OCT4, NANOG, TRA-1-60, TRA-1-81 and AKP. Nuclei staining with DAPI were in the lower right corner. Scale bar = 100 μm; (E) *ch*HES-480 has a normal karyotype 46, XY; (F) Derivatives from embryoid bodies of *ch*HES-480 cells could differentiate into ectoderm (TUJ1), mesoderm (SMA) and endoderm (AFP) *in vitro*. Scale bar = 50um; (G) Histological sections of teratomas formed by the *ch*HES-480 cell line contain tissues derived from three germ layers. Scale bar = 50um.

These embryos were cultured in G-series sequential medium (G1 and G2, Vitrolife) to the blastocyst stage at 37 °C in 5% CO2, 5% O2 and 90% N2 with 95% humidity.

hESC line derivation and cell culture

The chHES-480 cells were established in our laboratory as previously described (Lin et al., 2009). Briefly, the inner cell mass was mechanically isolated and was plated on the mouse embryonic fibroblast (MEF) feeders. The cells were cultured in serum-free DFSR medium, containing knock-out DMEM/F12 medium supplemented with 15% serum replacement, 0.1 mM β-mercaptoethanol, 1% nonessential amino acids, 2 mM L-glutamine, and 4 ng/mL human recombinant basic fibroblast growth factor (bFGF) (all from Invitrogen). Once the embryo outgrowth was observed, it was transmitted onto fresh feeders and obtained the hESC-colony morphology during prolonged culture. HESC colonies were passaged mechanically per week for long-term culture.

PCR-Sanger sequencing

Genomic DNA was prepared with QIAmp® DNA mini kit (QIAGEN) according to the manufacturer's instructions. Mutation of ABCD1 gene was examined by PCR amplification. Following primers were used for

PCR amplification reaction: 5′-GCAGAGTATCTTGGGGGA-3′ and 5′-GGTGGGACAAAGGGTGGC-3′. PCR was performed using 50 μL PCR amplification reaction mixture containing 1.5 μL of 100 ng genomic DNA, 1 μL of 1.0 μM of each primer, 25 μL of 2 × GreenMaster Mix (Promega) and 22.5 μL H_2O. Cycling conditions were as follows: 95 °C for 5 min followed by 35 cycles of amplification (95 °C denaturation for 40 s, 62 °C annealing for 40 s, 72 °C elongation for 40 s) and a final extension at 72 °C for 5 min. The products of PCR were determined by Sanger sequencing by using a BigDye Terminator cycle sequencing kit v3.1 and an ABI 3130XL Genetic Analyzer (Applied Biosystems).

RT-PCR

Total RNA was extracted with Trizol reagent (Invitrogen) and two microgram of RNA was reverse-transcribed into first-strand cDNA by using Transcriptor First Strand cDNA Synthesis Kit (Roche). The cDNA was submitted to RT-PCR using the primer pairs as described previously (Xie et al., 2016). PCR reactions were performed with GoTaq polymerase (Promega) on 0.2 μL of a reverse-transcription reaction mix in a total reaction volume of 10 μL. The PCR reaction was carried out as follows: 95 °C for 2 min followed by 35 cycles of amplification (95 °C for 30 s, 54– 64 °C for 30 s and 72 °C for 30 s), and a final extension at 72 °C for 5 min. PCR products were separated by electrophoresis on a 1.5% agarose gel, and images were taken on a UV transluminator.

Immunocytochemical staining and alkaline phosphatase staining

*Ch*HES-480 cells were harvested and fixed in 4% paraformaldehyde (PFA) for 20 min, and then blocked with 10% donkey serum in PBS for 30 min and incubated with primary antibodies overnight at 4 °C. For intracellular antigen, cells were blocked and permeabilized with PBS containing 0.2% Triton X-100 (Sigma) and 10% donkey serum (Jackson Immuno Reserach) for 30 min at room temperature. The following primary antibodies were used: mouse anti-OCT4 (1:200, Santa Cruz Biotechnology), rabbit anti-NANOG (1:100, Abcam), mouse anti-TRA-1-60 (1:50, Millipore), mouse anti-TRA-1-81 (1:50, Millipore), mouse anti-β-tubulin (1:800, Sigma), mouse anti-AFP (1:500, Sigma), and mouse anti-SMA (1:100, Millipore),overnight at 4 °C. Next, the cells were stained with Alexa Fluor® 488 donkey anti-mouse lgG (1:1000, Life Technologies) or Alexa Fluor® 488 donkey anti-rabbit lgG (1:1000, Life Technologies) for 1 h at room temperature in the dark. Nuclei were then counterstained with 4′, 6-diamidino-2-phenylindole (DAPI, 1:1000, Sigma). In addition, alkaline phosphatase activity was detected according to the protocol of the BCIP/NBT Kit (Invitrogen). Images were acquired in an epifluorescence microscopy (Nikon Eclipse TE2000-U).

Karyotyping analysis

*Ch*HES-480 cells were cultured in mTeSR™1 medium (STEMCELL) for 3-5 days, and then treated with 0.1 μg/mL of KaryoMAX® Colcemid™ solution (Life Technologies) for 3 h. After washing with PBS for three times, the cells were dissociated with Accutase (Millipore) into single cells and harvested using standard procedures, followed by standard G-banding for karyotyping. At least 20 metaphase spreads were examined for each sample using an Olympus epi-fluorescence microscope BX51 (Olympus, Tokyo, Japan) with LUCIA KARYOTYPE software (Lucia, Praha, Czech Republic).

In vitro and in vivo differentiation assay

For spontaneously *in vitro* differentiation assay, hESCs were mechanically dissociated into small clumps and cultured as aggregates in suspension for 7 days to form embryoid bodies (EBs) in DFSR medium without bFGF. Then EBs were transmitted onto Matrigel (BD) coated 4-well plates for another 14-day culture. The derivatives of embryoid bodies were processed for immunocytochemical staining analysis with three germ layer marker (Ectoderm: TUJ1; mesoderm: SMA; endoderm: AFP).

For *in vivo* differentiation assay, 1×10^6 hESC cells were injected intramuscularly into the right hind limb of 6-8-week-old mice with severe combined immunodeficiency disease (SCID) mouse. After 10 weeks, teratoma was harvested and fixed with 4% Paraformaldehyde. To identify the derivatives from three germ layers, hematoxylin and eosin (H&E) staining was carried out for the histological analysis. Animal work was carried out in the animal facility of Xiangya School of Medicine, Central South University in accordance with institutional guidelines.

Verification and authentication

Karyotyping and sequencing analyses were performed at Reproductive & Genetic Hospital of CITIC-Xiangya. Twenty metaphase cells were observed and all had a normal karyotype of 46, XY. After locus specific PCR, Sanger sequencing analysis confirmed a hemizygous missense mutation c.1825G>A(p.Glu609Lys) of *ABCD1* gene in *ch*HES-480 cells.

Acknowledgments

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